Book 13856 Page [redacted]5 Project No. 44300 Project Page 001

Investigator William McClements Date 9Oct97

Subject HPV L2 proteins

Filed in Book Number/Title WbM XVIII / wlm 97.14

9Dec97 Design PCR primers to allow generation of L2 vectors for testing directly in VLP or for fusing with E2 proteins for testing VLPs

1. will introduce unique NotI, SacI, and XhoI sites in L2 (in pGal11D HPV16 L1+L2)

a. See attached material for evaluation of strategy

b. PmlI use is may be dangerous, pGal HPV16 L1+L2 appears partially "nicked" by PmlI (see JCL)

2. primers ordered 9.10.97

MIDLAND FAXORDERFORM

Billing Information: Customer #40 Shipping Information: Deliver To end user

End-User Name: William McClements Phone 215 652 7861 FAX (7320)
WP26-251
Merck Research Laboratories 8 Oct. 1997
West Point, Pa. 19486

Ordering Person: William McClements Phone 215 652 7861

P.O. Number to be generated Purity DRY EP GF AE PAGE Other ____

Ship Day M T W T F S any UPSB FDX USM Quantity 1 2 3 5 10 other ODU all

13856-233a 47mer
5' CCT ACC GGC GGC CGC GAG CTC GAG GGT TAT ATT CCT GCA AAT ACA AC

13856-233c 27mer
5' GGG ACC GCG GCC GCC TGT ACC CGA CCC

13856-233d 35 mer
5' CCC TCC AGA CTC CTA GGC AGC CAA AGA GAC ATC TG

13856-233f 41 mer
5' GAC TAC GGC GGC CGC GAG CTC ACT GGG TAT ATT CCA TTG GG

13856-233g 33 mer
5' CAC GAC CTC GAG AGA TGT AGA GGG TAC AGA TGG

13856-233h 41mer
5' CGC CGC GGC GGC CGC GAG CTC CCA GCA TTA ACC TCT AGG CG

13856-233i 28 mer
5' CT TCC CCC CGG GCA CAA AAC AAA ATG C T

with f/c 9Dec97

Countersigned by _____ Date _____

MERCK & CO., INC./MRL
A49765 (10/77)

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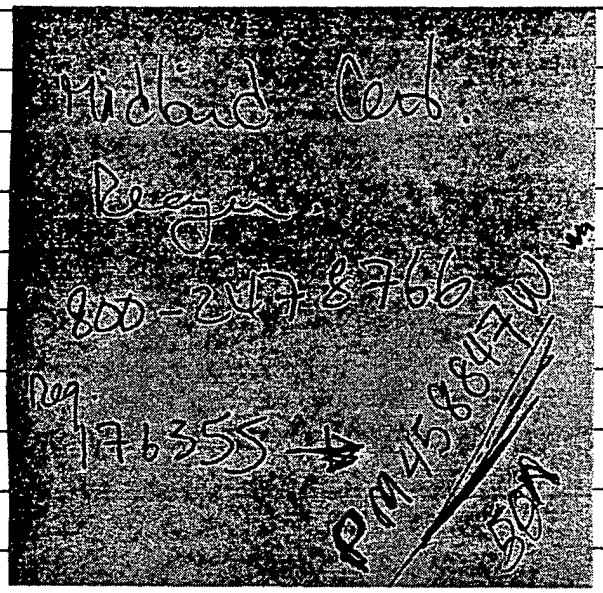
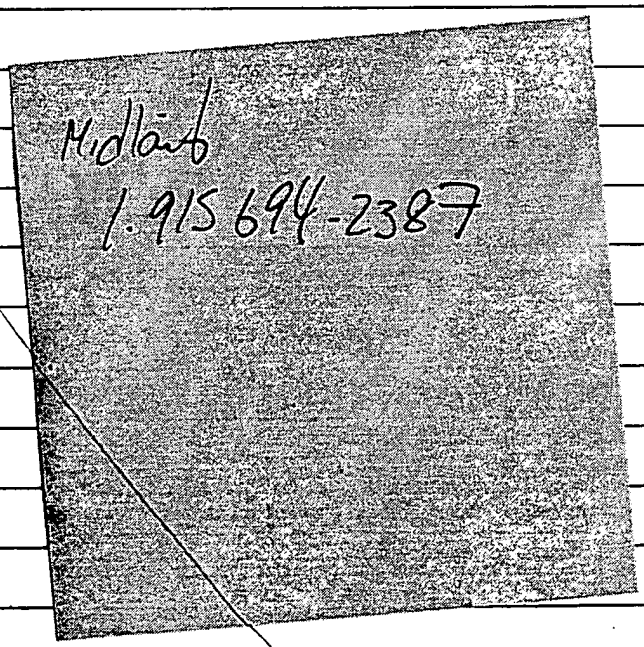




Witnessed by Arthur Friedman Date 5-20-98

Book 16344 Page 19 Project No. 44300 Project Page

Investigator Jessica Ling Date 15 Oct 97

Subject PCR of N-Terminus + C-Terminus of HPV16 L2

Filed in Book Number/Title Ling HPV-1 / 97-7 ~~and 97-8~~ JCL 16 Oct 97

Objective: to amplify via PCR the N-terminus and C-terminus of HPV16-L2 for creation of constructs. Refer to attachments for primer location & schematic.

15 Oct 97 Dilute primers from Midland. See accompanying pages for Certificates of Analysis. Resuspend w/ dH_2O

Dilute to 100 µM = $\frac{100 \times 10^{-6} \text{ moles}}{L} = \frac{100 \times 10^{-6} \text{ moles}}{106 \text{ µL}}$

$= 100 \times 10^{-12}$ moles/µL RCM66 #MI-7113

# 13856-233A	56.3 nmoles	= 563 µL
# 13856-233C	73.2 nmoles	= 732 µL
# 13836-233D	89.3 nmoles	= 893 µL
# 13836-233F	94.1 nmoles	= 941 µL
# 13836-233G	57.9 nmoles	= 519 µL
# 13836-233H	53.9 nmoles	= 539 µL
# 13836-233I	54.0 nmoles	= 540 µL

These are 100 µM stocks

Working stocks are 5 µM (1:20) w/ dH_2O

Set up pcr to amplify I+C and A+D fragments.

Component [Final]	1x Volume	3.5x Volume
D125-DNA [0.1 ng/λ]/50	1 µL	3.5 µL
10x Buffer [1x]	5 µL	17.5 µL
25 mM $MgCl_2$ [1.5 mM $MgCl_2$]	3 µL	10.5 µL
10 mM dNTPs [0.2 µM ea]	4 µL	14 µL
5 µM primer ① [0.2 µM]	2 µL	—
5 µM primer ② [0.2 µM]	2 µL	—
taq gold [2.5 U/rxn]	0.5 µL	1.75 µL / aliquot
dH_2O	32.5 µL / 50 µL	113.75 µL / 46 x / tube

Countersigned by Paulette Midgett Date 22 Oct 97

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

tube #

① Tm of #233 I = 66°C } anneal @ 58°C
#233 C = 60°C } product ~266bp

② Tm of #233A = 60°C } anneal @ 58°C
#233D = 60°C } product ~ 283bp

③ everything except primers. instead add
4µl dH_2O

Method for 9600 machine

① 95°C - 10' #55

② 95°C - 20"
58°C - 20" } 35X } #56 #59
72°C - 40"

③ 72°C - 10' #57

④ 4°C - hold #4

Component for PCR

taq gold - perkin elmer #G1092

25mM $MgCl_2$ - perkin elmer # G0873

10 x Buffer - perkin elmer # G0874

10mM dNTPs - GibcoBRL # JFT703

dH_2O - RCM66 # M1-7113

Jessica [signature]
16 Oct 97

Countersigned by Paulette Midgett Date 22 Oct

Book 16344 Page 257 Project No. [redacted] Project Page

Investigator Jessica Ling Date 03Dec97

Subject Seq Rxn's for P3 and Z7

Filed in Book Number/Title Ling Hpr-1 / JCL97113

Set up for Seq rxns for P3 and Z7.

P3 [1.2ug/λ] 1:4.8 = 2ul DNA + 7.2ul dH2O

Z7 [0.3ug/λ] 1:1.2 = 8ul + 1.6ul

Setup	M13	16A11
DNA	3ul	3ul
primer	1.1ul	3.2ul
taq FS	4ul	4ul
dH2O	11.9ul	9.8ul

Also sequence	M13/dH2O	16A11/dH2O
Z3 [0.18ug/λ]	4.2ul / 10.7ul	8.6ul / 4.2ul
Z4 [0.166ug/λ]	4.4ul / 10.5ul	8.4 / 4.4ul
P6 [0.187ug/λ]	3.9ul / 11ul	8.9 / 3.9ul
P7 [0.194ug/λ]	3.9ul / 11ul	8.9 / 3.9ul

Jessica Ling
03Dec97

Countersigned by Cornelia D. [illegible] Date 22-Jan-98

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

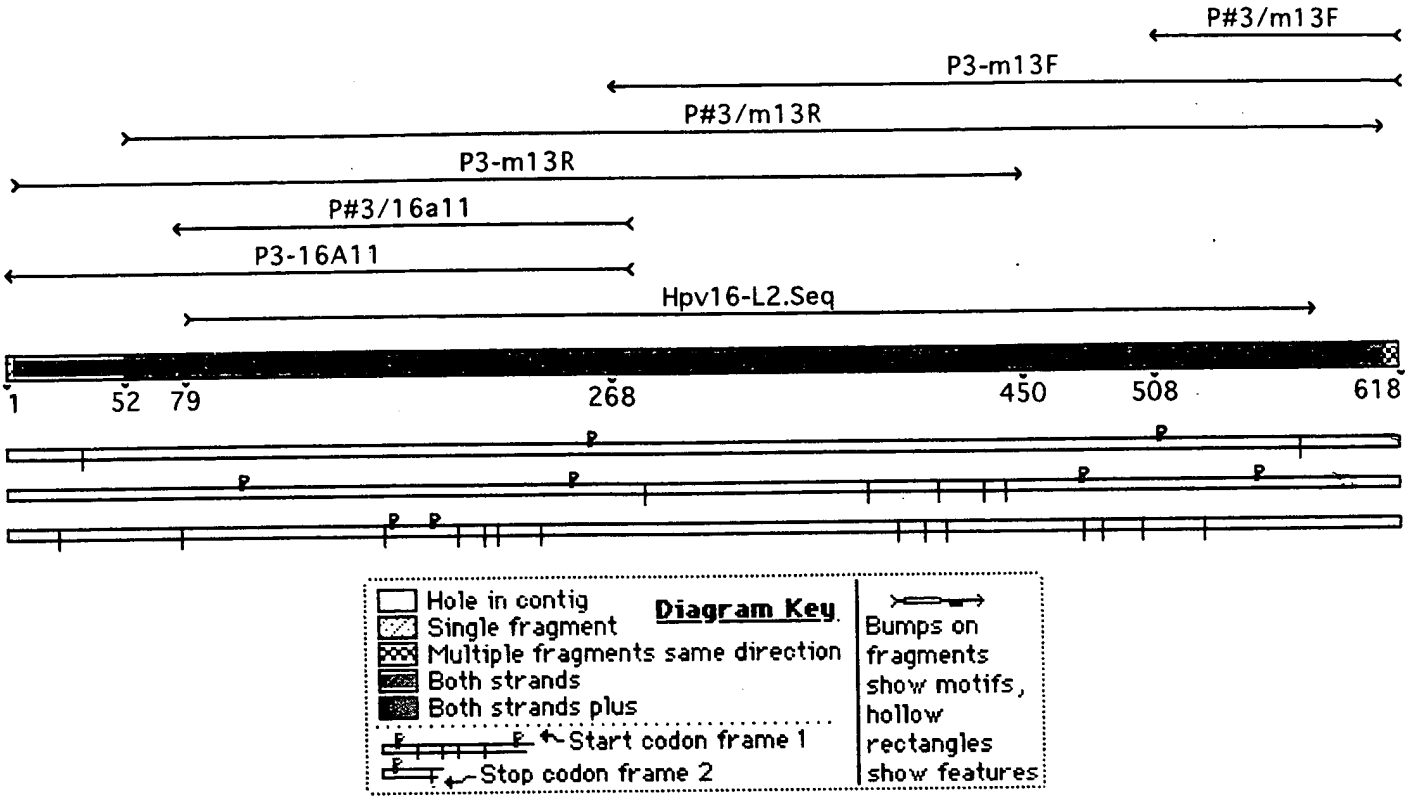
Results:
Still the primers as in the case the last time did not extend very well. But P3 was completely sequenced in two directions. So will continue with this clone
Also 27 was completly sequenced. Will also continue with this clone.

Both will be transformed into the yeast vector

Jessica
03Dec93

Countersigned by Connie De Bolt Date 22-Jan-94

P#3 Clone
Sequencher™ "Min. vector"



P#3 Clone
Sequencher™ "Min. vector"

P#3/m13R						TCCACCGC	GGTGGCGGCC
P3-m13R	ACGCCA	AGCGCGCAAT	TAACCCTCAC	TAAAGGGAAC	AAAAGCTGGA	GCTCCACCGC	GGTGGCGGCC
P3-16A11	GATTACGCCA	AGCGCGCAAT	TAACCCTCAC	TAAAGGGAAC	AAAAGCTGGA	GCTCCACCGC	GGTGGCGGCC
#1							
	GATTACGCCA	AGCGCGCAAT	TAACCCTCAC	TAAAGGGAAC	AAAAGCTGGA	GCTCCACCGC	GGTGGCGGCC

P#3/m13R	GCTCTAGCCC	CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG
P3-m13R	GCTCTAGCCC	CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG
P#3/16a11	AGCCC	CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG
P3-16A11	GCTCTAGCCC	CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG
Hpv16-L2.Seq		CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG
#71							
	GCTCTAGCCC	CTTCCCCCCG	GGCACAAAAC	AAAATGCGAC	ACAAACGTTC	TGCAAAACGC	ACAAAACGTG

P#3/m13R	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTTCA	CCTGACATTA	TACCTAAGGT
P3-m13R	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTCCA	CCTGACATTA	TACCTAAGGT
P#3/16a11	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTCCA	CCTGACATTA	TACCTAAGGT
P3-16A11	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTCCA	CCTGACATTA	TACCTAAGGT
Hpv16-L2.Seq	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTCCA	CCTGACATTA	TACCTAAGGT
#141							
	CATCGGCTAC	CCAACTTTAT	AAAACATGCA	AACAGGCAGG	TACATGTCCA	CCTGACATTA	TACCTAAGGT

P#3 Clone
Sequencher™ "Min. vector"

```
P3-m13F                                                                    TTTTTGG TGGGTTAGGA
P#3/m13R      TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTGG TGGGTTAGGA
P3-m13R       TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTGG TGGGTTAGGA
P#3/16a11     TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTG: TG
P3-16A11      TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTG: TG
Hpv16-L2.Seq  TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTGG TGGGTTAGGA
    #211      ..........................................................................
              TGAAGGCAAA ACTATTGCTG ATCAAATATT ACAATATGGA AGTATGGGTG TATTTTTTGG TGGGTTAGGA
                                                                                •

P3-m13F       ATTGGAACA: GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAATACA ACAATTCCTT
P#3/m13R      ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAATACA ACAATTCCTT
P3-m13R       ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAATACA ACAATTCCTT
Hpv16-L2.Seq  ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAATACA ACAATTCCTT
    #281      ..........................................................................
              ATTGGAACAG GGTCGGGTAC AGGCGGCCGC GAGCTCGAGG GTTATATTCC TGCAAATACA ACAATTCCTT
                    •

P3-m13F       TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
P#3/m13R      TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
P3-m13R       TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG AACAAGCTCC
Hpv16-L2.Seq  TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
    #351      ..........................................................................
              TTGGTGGTGC ATACAATATT CCTTTAGTAT CAGGTCCTGA TATACCCATT AATATAACTG ACCAAGCTCC
                                                                                •
```

P#3 Clone
Sequencher™ "Min. vector"

```
P3-m13F          TTCATTAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTTATTTA
P#3/m13R         TTCATTAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTTATTTA
P3-m13R          TTCATTAATC CTATAGTTC CAGG
Hpv16-L2.Seq     TTCATTAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTTATTTA
      #421
                 TTCATTAATT CCTATAGTTC CAGGGTCTCC ACAATATACA ATTATTGCTG ATGCAGGTGA CTTTTATTTA

P#3/m13F                    ACATGTT ACGAAAACGA CGTAAACGTT TACCATATTT TTTTTCAGAT GTCTCTTTGG
P3-m13F          CATCCTAGTT ATTACATGTT ACGAAAACGA CGTAAACGTT TACCATATTT TTTTTCAGAT GTCTCTTTGG
P#3/m13R         CATCCTAGTT ATTACATGTT ACGAAAACGA CGTAAACGTT TACCATATTT TTTTTCAGAT GTCTCTTTGG
Hpv16-L2.Seq     CATCCTAGTT ATTACATGTT ACGAAAACGA CGTAAACGTT TACCATATTT TTTTTCAGAT GTCTCTTTGG
      #491
                 CATCCTAGTT ATTACATGTT ACGAAAACGA CGTAAACGTT TACCATATTT TTTTTCAGAT GTCTCTTTGG

P#3/m13F         CTGCCTAGAG ATCTGGAGGG CGGATCCCCC GGGCTGCAGG AATTCGATAT CAA
P3-m13F          CTGCCTAGAG ATCTGGAGGG CGGATCCCCC GGGCTGCAGG AATTCGATAT CAA
P#3/m13R         CTGCCTAGAG ATCTGGAGGG CGGATCCCCC GGGCTGCAGG AATTC
Hpv16-L2.Seq     CTGCCTAGAG ATCT
      #561
                 CTGCCTAGAG ATCTGGAGGG CGGATCCCCC GGGCTGCAGG AATTCGATAT CAA
```

Book 16344 Page 233 Project No. Project Page

Investigator Jessica Lung Date 25Nov97

Subject Ligation #12/Transformation

Filed in Book Number/Title Lung HPV-1 / JCL97-103

Set up ligation #12

Another attempt for a 3 way ligation into the yeast vector

I/C' (SmaI/NotI) JCL97-59

A'/D2 (NotI/BglII) JCL97-96

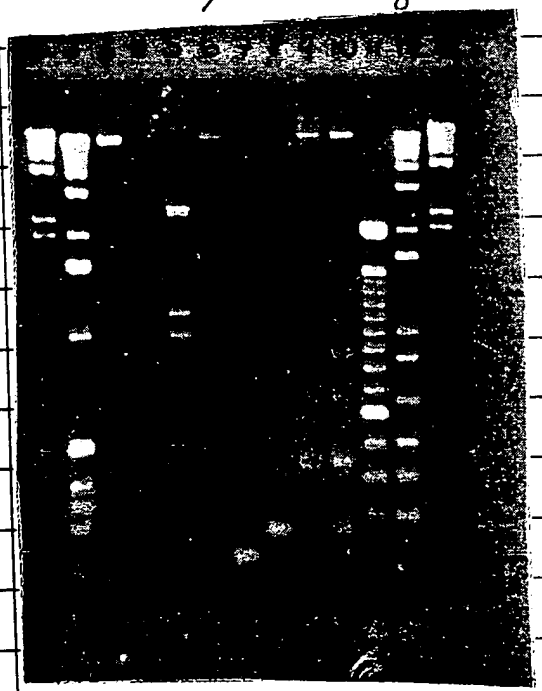
TUBE #	Vector Name	# µL	Insert Name	# µL	10 x Buffer	T4 DNA Ligase	# µL dH2O
1	D03(SmaI/BglII)	1ul	~~⊖~~	~~⊖~~	2ul	1ul	16ul
2			VIN:AgB3(PCI)	2			14ul
3			I/C', A'/D2	4/4			8ul
4			I/C'/A'/D2	6/6			4ul

Incubate O/N @ 16°C

26Nov97 PCI of ligation, EtOH ppt. (BMB # 83628520)

Resuspend into 20µl dH2O

load 10µl onto gel



1. λHind3 5ul
2. 1Kbladder 5ul
3. D-03 lig 1ul
4. Lig ①
5. VIN:AgB-3 1ul
6. Lig ②
7. I/C' (SmaI/NotI) 2ul
8. A'/D2 (NotI/BglII) 2ul
9. ③ lig
10. Lig ④
11. 100bp ladder 5ul
12. 1Kb ladder 5ul
13. λHind3 ladder 5ul

Jessica Lung 26Nov97

Countersigned by Connie Digilio Date 22-Jan-98

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A49765 (10/77)

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Transform into DH5 (# JJB703 Gibco) 25ul c

① 24

② ~1-200 colonies

③ 30µl = 13 70µl = 39

④ 1 = 14 = 32

30µl of total 100µl transformatn

70µl of 100µl transformatn

Plated onto Remel LBAMp #5639 exp 12-1

Pick + patch colonies.

Jessica R
26Nov97

Bk 16344 Page 248 Project No. Project Page

Investigator: Jessica Ling Date 02Dec97

Subject Pre-screen of JCL97-103

Filed in Book Number/Title Lig HPV-1 / JCL97-110

Set up clonal screen (96) of JCL97-103 (3way lig.)

COMPONENT	Manufact/Lot Number	[FINAL]	1 X VOLUME	? X VOLUME
				110x
DNA -- Clones			20µL	—
10 x Buffer	Perkin Elmer/ #G0874	1 X	5µL	550ul
25mM MgCl2	Perkin Elmer/ #G0873	3.0mM	6µL	660ul
10mM dNTPs	Stratagene/ #0276151	0.8µM	4µL	440ul
5µM Primer #1	233 I	0.2µM	2µL	220ul
5µM Primer #2	233 D2	0.2µM	2µL	220ul
Taq Gold	Perkin Elmer/ #G109[illegible]	1.25U	0.2µL	27.5ul
dH2O	RCM66/ #M1-7113		10.73ul	1182.5ul
Total			50µL	Aliquot 30µL/Tube

JCL 02Dec97

~~Rxn~~ Method:

① 95°C - 10'

② 94°C - 45" } 30X
60°C - 45" }
72°C - 45" }

③ 72°C - 10'

④ 4°C hold

Results:

Positives # (10/96)

	(11)	75
	26	(79)
	(29)	(81)
	(44)	84
	71	95

Circled clones will be grown up in 100ml for DNA prep

Jessica L.
02Dec97

Countersigned by Connie de Hall Date 22-Jan-98

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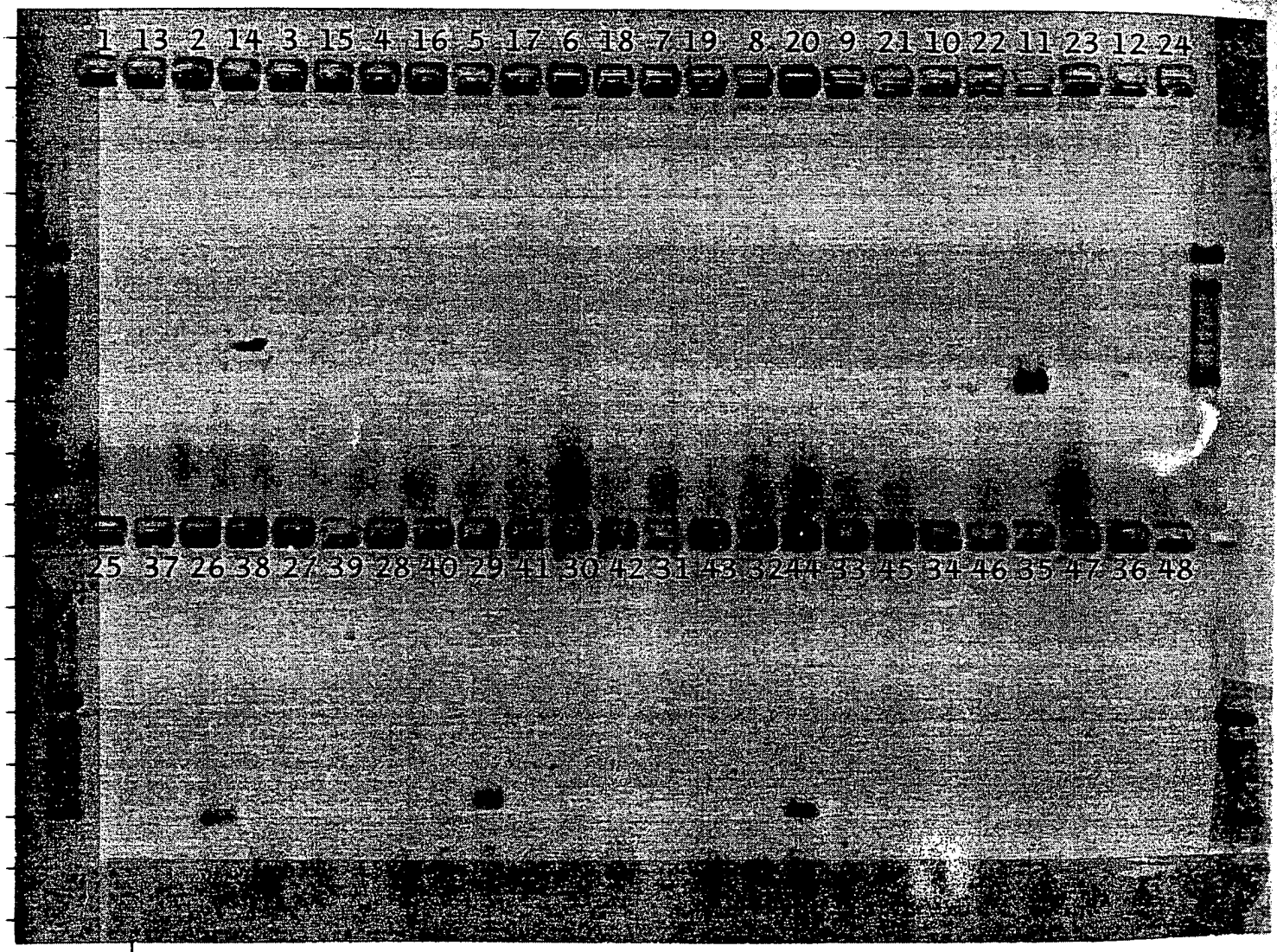
JCL 97•110
3-Way Ligation Colony Screen
(pGAL110) 3WID#1-48



Jessica Ly
02Dec97

Countersigned by Connie DeSilva Date 22 Jan 98

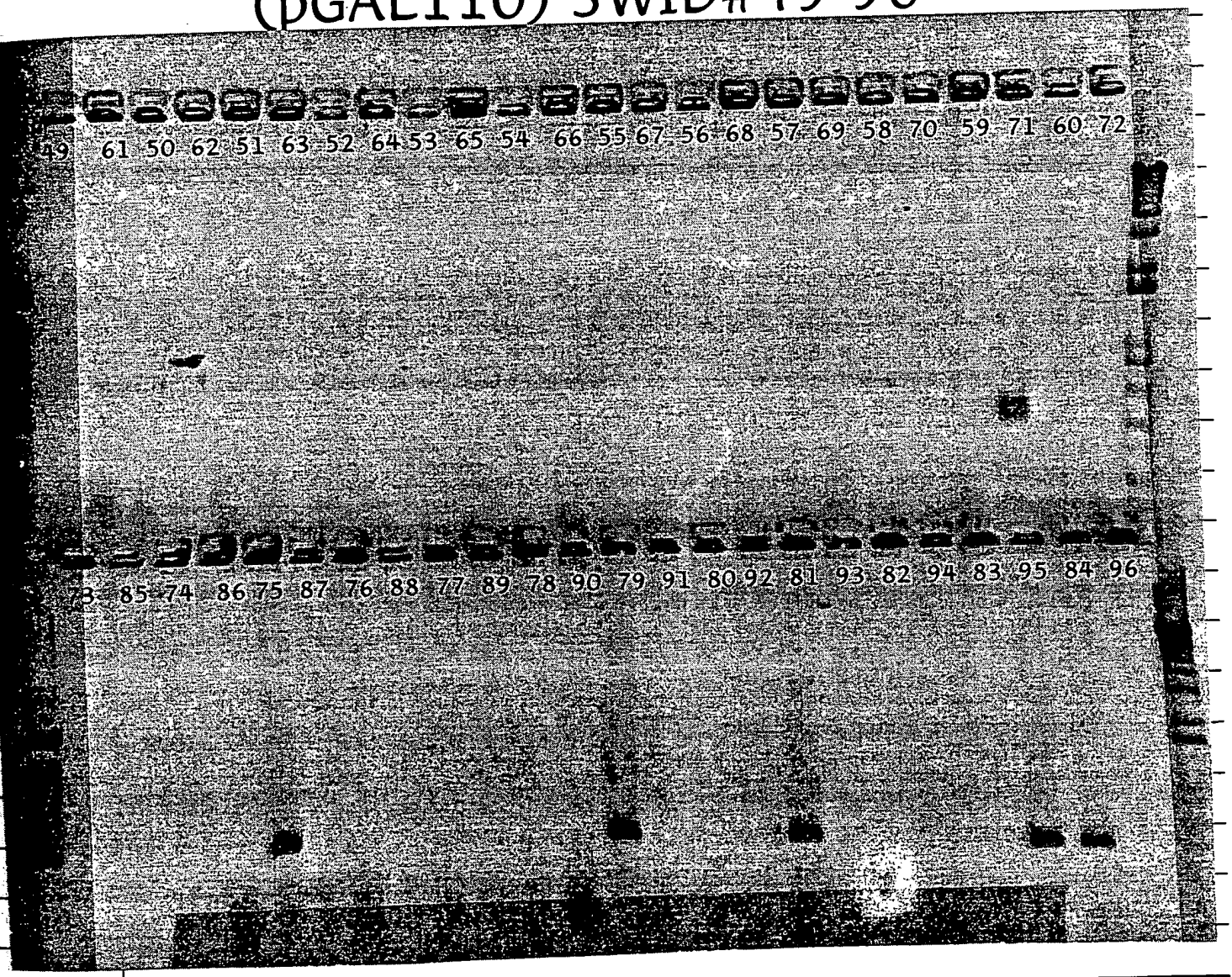
Book 16344 Page 251 Project No. _____ Project Page _____

Investigator Jessica Ling Date 02Dec97

Subject Colony screen JCL97-103

Filed in Book Number/Title Ling HPV-1 / JCL97-110 Continued

JCL 97•110
3-Way Ligation Colony Screen
(pGAL110) 3WID#49-96



Jessica Ling
02Dec97

Witnessed and understood by Corrie DeWitt Date 2-Jan-98

Book 10038 P E257 Project No. [redacted] Project Page

Investigator [signature] Date Oct 9, 1997

Subject Mutagenesis PCR amplification of 16E2 from HPV

Filed in Book Number/Title HPV4

Purpose: Express 16E2 in yeast linked to L2 want to see if incorporated into VLPs. Must first knock out 2 amino acids responsible for E2 function

Materials: "Wild type 16E2" Clone 1415 from J. Zhang
Oligos - See E257A for what was ordered & changes made to Gene

Methods: Reference paper for Mutants = J Virology Vol 70 (3) 1602-1611 Sakai, Hiroyuki; et al

PCR out inserts as 3 PCR products

	LA Taq ①	LA Taq ②	LA Taq ③	AmpliTaq (BMB) ④	AmpliTaq (BMB) ⑤	AmpliTaq (BMB) ⑥
H_2O	83.5	83.5	83.5	83.5	83.5	83.5
10x buffer + Mg	10	10	10	10	10	10
25 mM dNTP	2	2	2	2	2	2
Primer: Top 100pm/λ	1.5 MN492	1.5 MN494	1.5 MN496	1.5 MN492	1.5 MN494	1.5 MN496
Bottom 100pm/λ	1.5 MN493	1.5 MN495	1.5 MN497	1.5 MN493	1.5 MN495	1.5 MN497
Template	1	1	1	1	1	1
Taq Pol. 5U/λ	0.5	0.5	0.5	0.5	0.5	0.5
	100	100	100	100	100	100

#'s 4-6 get BMB 10x Buffer; Boehringer Mannheim Taq

94°C, 1 min
94°C, 30"
50°C 30"
68°C 30"
] ~~20~~ 18 cycles MN Oct 9, 1997
linked +4°C Overnight

Note All Rxns performed in duplicate

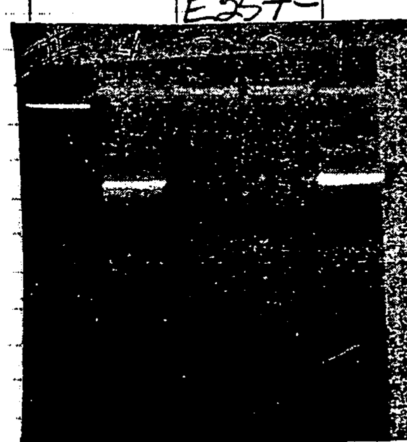
XhoI NaeI Not — Xho Nae Not

Note: Must blunt end this in not to destroy sites.

[signature] 10/9/97

[signature] 10/10/97

E257



100 bp ladder

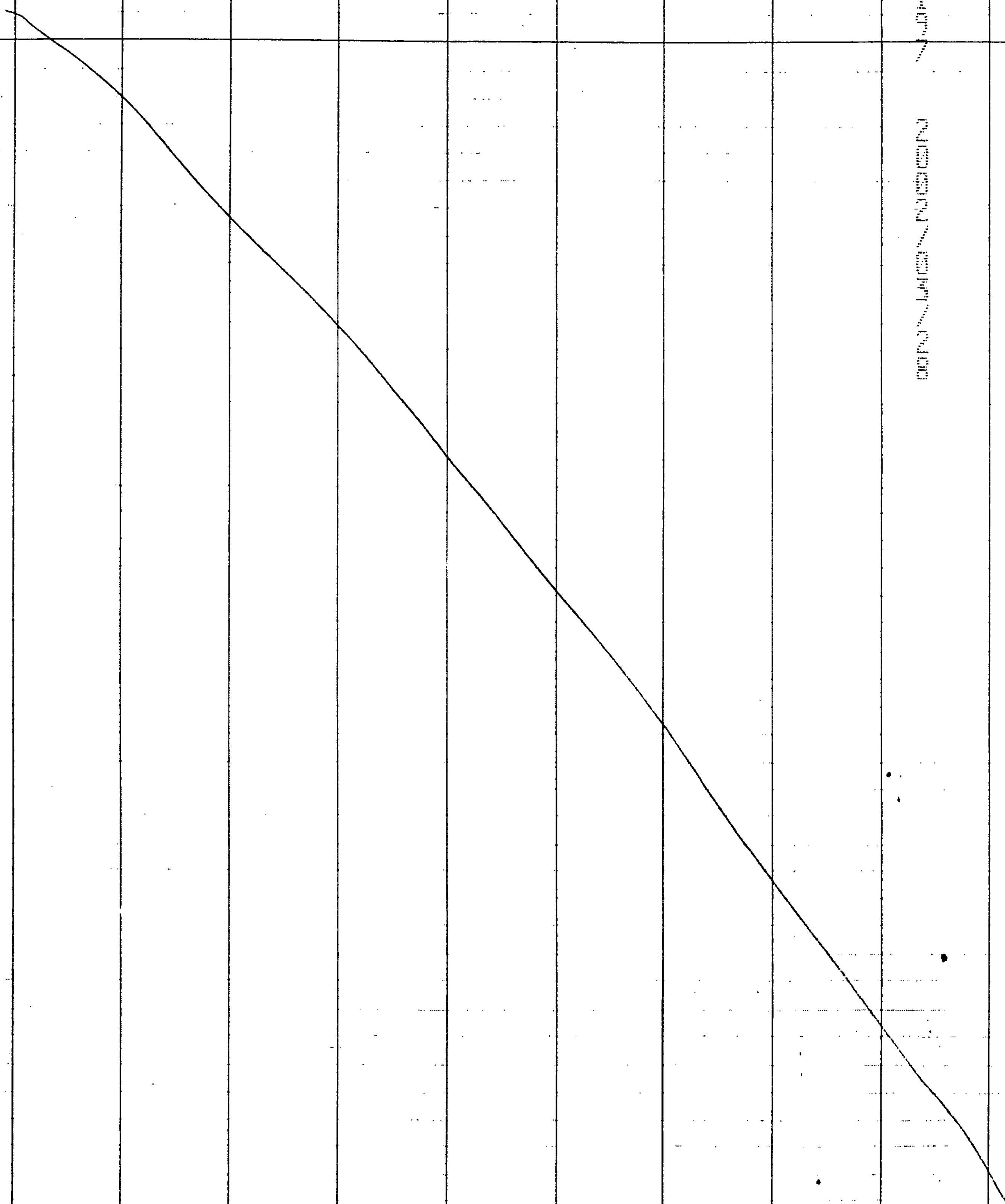
Results:
See gel - Should be
#1 = 160 bp
#2 = 135 bp
#3 = 900 bp
#4 = (same as #1 but using Taq Pol.

Countersigned by [signature] Date 2/3/98

L-1003 (11/78)
MERCK & CO., INC./MSDRL

Book 10038 Page 258 Project No. ____ Project Page ____

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.



(8)

Countersigned by ____ Date ____

Book 10038 ge E295 Project No. [redacted] Project Page

Investigator [redacted] Date 12/11/97

Subject Insertion of Dead 16Ez Mutant into 16Lz (minimal) expression

Filed in Book Number/Title HPV4

Purpose: Insert 16Ez of dead mutant (E280) into Lz for expression and to see if incorporated into VLPs.

Materials:

Received YP3-1 (yeast pGAL110 HPV16 L1, Lz minimal vector) from G. Ling.

E280-6 NotIXhoI frag from This notebook
E280-8 " " "

Methods:

NotI, XhoI Digest

	(1) = YP3 Vector	(2) = E280-6	(3) = E280-8
✓ DNA	10	20	20
✓ 10x H buffer	10	10	10
✓ H_2O	70	60	60
XhoI	5	5	5
	95	95	95
37°C, 30 min NotI	5	5	5
	100	100	100

37°C ~ 2½ hr

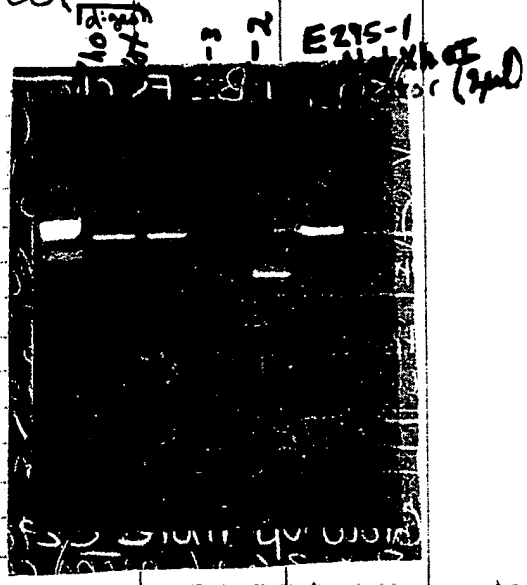
Gel is on 1% LMP Agarose (Inserts only)

Vector: Ethanol ppt.

Shrimp alkaline phosphatase treatment
E295-1 Not Xho digest

44 µl H_2O (DNA)
5 µl 10x buffer
0.5 µl Shrimp Alk Phos.
50

37°C 1 hr.



Phenol extract -1 -2 & -3 1x CHCl3 extract 1x and Eth. ppt
Resuspend in 20µl H_2O Run on gel above ↑

Ligate as Reverse (E296)

L-1003 (11/78)

Countersigned by Beth A Arnold Date 2/3/98

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Ligation:

	(1)	(2)	(3)
vector 41F3-1 Not XhoI shrimp AKPhos	5	5	5
insert	4 (E295-2)	4 E295-3	0
10x lig. buffer BMB	1.5	1.5	1.5
H_2O	3.5	3.5	7.5
T4 DNA ligase	1	1	1
	15	15	15

15°C O/N

Transform DH5 cells as usual (BRL Comp. cells) plate LB Amp Oker Wknd @ 28 C

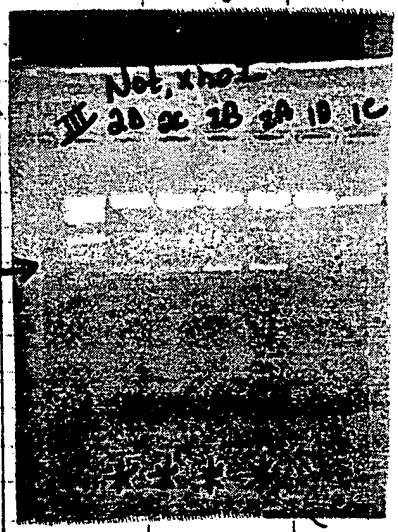
Emp. Helper 12/19/97

Pick colonies from ligation rxns E296-1 & E296-2 lig since the colony count was so high relative to the no insert control ligation plates (approx. 5-10x more cfus on -1 & -2 vs. -3)

Pick 4 clones from each plates E296-1 & 2 (lost 2 clones during isolation isolate DNA from O/N cultures in LB Amp using Promega Mid prep SV (3.6 ml culture). Followed their procedure, did not ethanol ppt before sequencing.

Digest clones w/ Not XhoI to confirm presence of ~1.2 Kb 16Ez in E296-

Picked clones 2A & 2B for Sequencing Using ABI FS Chem. Terminator dye mix as standard using 6µl (of 100µl) DNA from each prep. See sections #14 & 15 of book #4 Sequencing & gel files on pZIP100 drive



~1.2 kb

correct clones.

Results:

E296-2B Sequence appears to be good with no changes that would affect AA translation.

Emp. Helper 12-19-97

Grow up more E296-2B, isolate using 8 x 3.6 ml preps of Promega Wizard SV Miniprep kit. Then pheno/CHCl3 extract, Ethanol ppt wash & dry Resuspend sterile water for yeast transformation

Give to B. McClements

Emp. Helper 12-19-97

Countersigned by Barbara Arnold Date 2/3/98

Book 10038 Page E.297 Project No. _____ Project Page _____

Investigator [redacted] Date 12/15/97

Subject Insertion of 16E7 into YIB-1 Minimal L2 Vector

Filed in Book Number/Title HPV4

Purpose: Insert 16E7 into middle of 16L2 coding region of Minimal L2 orf.

Materials:

16E7 DNA from 1415 clone from J. Zhang

Methods:

PCR orf using primers MN512/MN513

	①	②	③	④
H_2O	40.5			
10x PCR buffer w Mg (BMB)	5			
10 mM dNTP's (BMB)	2			
Primer MN512 500 ng/μl	0.5			
MN513 500 ng/μl	0.5			
Taq Pol	0.5			
Template 1415 16E7	1.0			
	50			

Rxn in quadrup

94°C 1 min

94°C 30"
55°C 30"
72°C 30"
] 15 cycles.

7 min 72°C
4°C hold.

from the 4 rxns above pooled & run
Gel isolate 0.3 Kb frags on 1.8% LMP (SeaPlaque) agarose
Cut appropriate band and digest w/ Agarase (BMB) 45°C for 1.5 hr
Phend/$CHCl_3$ i ppt resusp. in ~~480~~ 160 μl H_2O use half in each of 2 tubes as belo

Not XhoI Digest (Done in 2 tubes)

DNA	80
10x "H" buffer	10
H_2O	05
Not I	
Xho II	5 MN 12/19/97
	100

37°C, 2 hr (tube 1)
37°C, Overnight (tube 2)

Phend/$CHCl_3$ ppt wash dry resuspend tube 1 in 20 μl H_2O
Use for ligation E298.

Countersigned by Beth A Arnold Date 2/3/98

L-1003 (11/78)

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

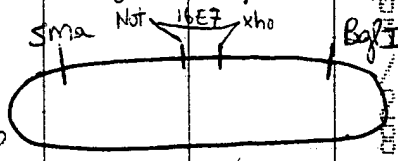
Ligation:

(1)

Vector: E295-1 NotI xhoI "Shrimped" 3
insert: E297-tube 1 NotI xhoI 16E7 3

BMB Reagents
10X lig buffer (w/ ATP) 1.2
H_2O 5
T4 DNA ligase 0.8
12.0

14°C O/N
Transform DH5 cells as usu. (See E296)
Pick 9 transformants, grow cultures overnight 37°C LB Amp 3.6 ml culture
Isolate DNA - Promega Wizard SV (Harvest) Resus. 100 µl H_2O



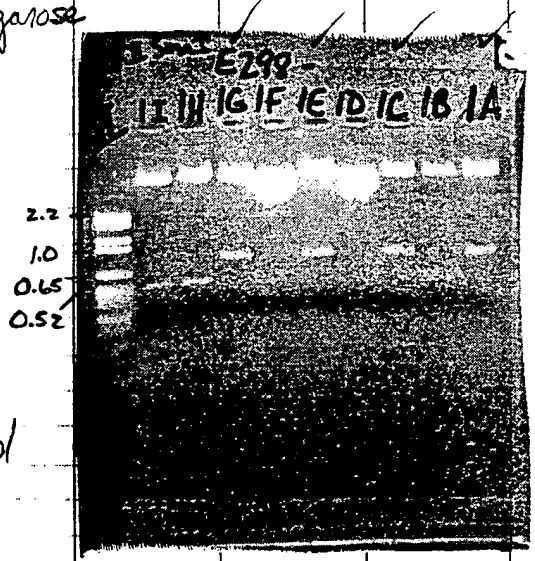
Analysis: BglII, SmaI digest.

Above digest would release a ~550 bp frag if no 16E7 was present or ~900 bp if 16E7 was located w/in BglII SmaI

PCR Conditions for Digest: MN 1/5/98
37°C, 30'; 25°C, 30'
Run on 1.2% Agarose

Digest:

DNA	7
10X Alby	2
H_2O	9
BglII	1
SmaI	1
	20



Correct Clones = E298-1A, 1C, 1E, 1G
Sequence w/ FS chem (ABI) Gel DNA
(See section 16 of Seq Book #4)

Results:

Sequence of clone E298-1G appears correct (2 other clones had changes and 1 other clone lacked good sequence reads in both directions)
(Primers from J. Ling - See seq book #4)

Isolate DNA from E298-1G using Promega Wizard SV prep then phenol CHCl3 Ethol ppt Wash dry. Resuspend in 30 µl sterile dH2O RCM66; Provide 25µl to McClements, W. for transformation of yeast

Jan 5, 1998

Countersigned by Beth A Arnold Date 2/3/98

Book 10038 Page E 299 Project No. _____ Project Page _____

Investigator [redacted] Date Dec 29, 9

Subject Preparation of 16E7 and Insertion into Gast L1 + L2 vector.

Filed in Book Number/Title HPV 4

Purpose: Inserted 16E7 orf into NotI XhoI of L1+L2 minimal vector (= construct E29
Now want to insert 16E7 into the full length L2 orf at NotI and Xh
sites.

Materials:

vectors (from J. Ling) YGH#3 (JCL97.131) Nuclear Localization Signal L
YFG#5 (JCL97.131) full length L2
TA vector - Invitrogen (for subcloning

insert: 16E7 template = 1415 from J. Zhang
primers = MN512 MN513 from Midland Reagent Co.

Method:

	1X
H_2O	40.5
10x Taq buffer w/ Mg (BMB)	5
10 mM dNTPs (BMB)	2
Primer: MN512	0.5
MN513	0.5
Template	1.0
Taq Pol 5U/x (BMB)	0.5
	50

(did 4 of these reactions for more final product)

Cycles: 94°C, 55°C, 72°C — 30" each — 15 cycles

7 min 72°C hold → 4°C ∞

Store -20°C

~~[redacted]~~ 12/29/97

~~[redacted]~~ 12/31/97

Gel separate 1.8% LMP Agarose thin gel iso by Agarose (BMB) Method
Resuspend 30 µl. = E299-1

Digestion:

Cut 1/3rd w/ XhoI (5U/µl) 1/3rd w/ NotI, 1/3rd Uncut

DNA	10	DNA	10
10x H buffer	7	10x H buffer	7
H_2O	46	H_2O	46 MN 12/31/97
XhoI	7	NotI	7
	70		70

37°C, 2 hr

Countersign d by Beth A Arnold Date 2/3/98

L-1003 (11/78)

DO NOT BEGIN NEW EXPERIMENTS ON THIS PAGE.

Vector Prep:

	YFG#5 (1)	YFG#5 (2)	YGH#3 (3)	YGH#3 (4)
DNA	15	15	15	15
10x H buffer	10	10	10	10
H_2O	65	65	65	65
Enzyme	10 NotI	10 XhoI	10 NotI	10 XhoII
	100	100	100	100

37°C, ~3 hr

Note: Since DNA appears viscous - I am doubling above volumes for each to 200µl by addition of: 10µl 10x buffer, 5µl more Enzyme, 8µl H_2O

Phenol·$CHCl_3$, ppt resuspend 44µl dH_2O

Shrimp Alk Phosphatase see E295 for Materials & Method of Use

DNA 44µl
10x buf 5
Shrimp Alk Phos 0.5
~50

Note: Doubled recipe here too to 100µl as this is large plasmid and at high conc/µl.

37°C 1 hr
Phenol $CHCl_3$ ppt Resusp. 30µl (See gel E299) NR5+

Full length

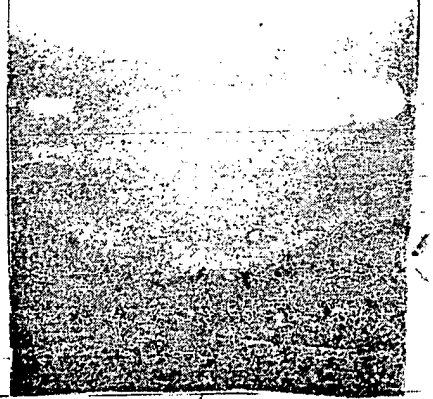
	YFG#5 vector A-(A)		YGH#3 vector C-(C)		LA vector E-(E)	(6)
Ligation:						
Vector	2 E300-1	2 E300-2	6 E300-3	6 E300-4	2 TA Vector (Invitrogen)	
insert	4 E299-1 Not	4 E299-1 Xho	4 E299-1 Not	4 E299-1 Xho	2 E299-1 UNCut	
10x lig buf	2	2	2	2	1	
H_2O	10	10	7	7	4	
T_4 DNA ligase	1	1	1	1	1	
	20	20	20	20	10	

E299-1 x E299-1 N → E300 4 3 2 1

Long Run E2 c

14°C until 1/13/98 — 5-Jan98

Transform Gibco DH5 cells per Manufact. Plate Remel LB Amp plates @ 37°C O/N — 5-Jan98



Countersign d by Beth A Arnold Date 2/3/98

Book 16344 Page 27[illegible] Project No. [illegible] Project Page

Investigator Jessica Rup Date 08Dec97

Subject Sequencing of YP3-1, 2, 70 and 72

Filed in Book Number/Title Ling HPV-2 / JCL97-118

Setup Sequencing Rxn for YP3 #1, #2, #70 & #72

Use: L2/184F as primer

L2/167R

L2/246bF

L2/157F

L2/45R

L2/1261R

L2/1384F

L2/1339F

L2/16A11

dilute DNA to [0.25ug/l]

DNA - 3ul

FStaq - 4ul

primer 5uM / 1pmol 1.1ul / 3.2ul

dH_2O 11.9ul / 9.8ul

Results:

Looked at YP3 #1 and YP3 #2 under analysis program. Both looked very good.

Will hand YP3 #1 to Bill McClements as sequenced vector. Also will give to Mike Neeper to clone E proteins.

Jessica Rup
10Dec97

Countersigned by Connie Desjarlais Date 06-Feb-98